

emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, or polymers.

Alternatively, the novel Cry1C-derived mutated crystal proteins may be prepared by native or recombinant bacterial expression systems *in vitro* and isolated for subsequent field application. Such protein may be either in crude cell lysates, suspensions, colloids, *etc.*, or alternatively may be purified, refined, buffered, and/or further processed, before formulating in an active biocidal formulation. Likewise, under certain circumstances, it may be desirable to isolate crystals and/or spores from bacterial cultures expressing the crystal protein and apply solutions, suspensions, or colloidal preparations of such crystals and/or spores as the active bioinsecticidal composition.

Another important aspect of the invention is a method of controlling lepidopteran insects which are susceptible to the novel compositions disclosed herein. Such a method generally comprises contacting the insect or insect population, colony, *etc.*, with an insecticidally-effective amount of a Cry1C\* crystal protein composition. The method may utilize Cry1C\* crystal proteins such as those disclosed in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:59, or SEQ ID NO:61, or biologically functional equivalents thereof. Alternatively, the method may utilize one or more Cry1C\* crystal proteins which are encoded by the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:58, or SEQ ID NO:60, or by one or more nucleic acid sequences which hybridize to the sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:58, or SEQ ID NO:60, under conditions of moderate, or higher, stringency. The methods for identifying sequences which hybridize to those disclosed under conditions of moderate or higher stringency are well-known to those of skill in the art, and are discussed herein.

Regardless of the method of application, the amount of the active component(s) are applied at an insecticidally-effective amount, which will vary depending on such factors as, for example, the specific lepidopteran insects to be controlled, the specific plant or crop to be treated, the environmental conditions, and the method, rate, and quantity of application of the insecticidally-active composition.

The insecticide compositions described may be made by formulating either the bacterial cell, crystal and/or spore suspension, or isolated protein component with the desired agriculturally-acceptable carrier. The compositions may be formulated prior to administration in an appropriate means such as lyophilized, freeze-dried, dessicated, or in an aqueous carrier, medium or suitable diluent, such as saline or other buffer. The formulated compositions may be in the form of a dust or granular material, or a suspension in oil (vegetable or mineral), or water or oil/water emulsions, or as a wettable powder, or in combination with any other carrier material suitable for agricultural application. Suitable agricultural carriers can be solid or liquid and are well known in the art. The term "agriculturally-acceptable carrier" covers all adjuvants, *e.g.*, inert components, dispersants, surfactants, tackifiers, binders, *etc.* that are ordinarily used in insecticide formulation technology; these are well known to those skilled in insecticide formulation. The formulations may be mixed with one or more solid or liquid adjuvants and prepared by various means, *e.g.*, by homogeneously mixing, blending and/or grinding the insecticidal composition with suitable adjuvants using conventional formulation techniques.

The insecticidal compositions of this invention are applied to the environment of the target lepidopteran insect, typically onto the foliage of the plant or crop to be protected, by conventional methods, preferably by spraying. The strength and duration of insecticidal application will be set with regard to conditions specific to the particular pest(s), crop(s) to be treated and particular environmental conditions. The proportional ratio of active ingredient to carrier will naturally depend on the chemical nature, solubility, and stability of the insecticidal composition, as well as the particular formulation contemplated.

Other application techniques, *e.g.*, dusting, sprinkling, soaking, soil injection, seed coating, seedling coating, spraying, aerating, misting, atomizing, and the like, are also feasible and may be required under certain circumstances such as *e.g.*, insects that cause root or stalk infestation, or for application to delicate vegetation or ornamental plants. These application procedures are also well-known to those of skill in the art.

The insecticidal composition of the invention may be employed in the method of the invention singly or in combination with other compounds, including and not limited to other pesticides. The method of the invention may also be used in conjunction with other treatments such as surfactants, detergents, polymers or time-release formulations.

5 The insecticidal compositions of the present invention may be formulated for either systemic or topical use.

The concentration of insecticidal composition which is used for environmental, systemic, or foliar application will vary widely depending upon the nature of the particular formulation, means of application, environmental conditions, and degree of 10 biocidal activity. Typically, the bioinsecticidal composition will be present in the applied formulation at a concentration of at least about 1% by weight and may be up to and including about 99% by weight. Dry formulations of the compositions may be from about 1% to about 99% or more by weight of the composition, while liquid formulations may generally comprise from about 1% to about 99% or more of the active ingredient by weight. Formulations which comprise intact bacterial cells will generally contain from about  $10^4$  to about  $10^{12}$  cells/mg.

The insecticidal formulation may be administered to a particular plant or target area in one or more applications as needed, with a typical field application rate per hectare ranging on the order of from about 1 g to about 1 kg, 2 kg, 5, kg, or more of active ingredient.

## 2.10 BIOLOGICAL FUNCTIONAL EQUIVALENTS

Modification and changes may be made in the structure of the peptides of the present invention and DNA segments which encode them and still obtain a functional 25 molecule that encodes a protein or peptide with desirable characteristics. The following is a discussion based upon changing the amino acids of a protein to create an equivalent, or even an improved, second-generation molecule. In particular embodiments of the invention, mutated crystal proteins are contemplated to be useful for increasing the insecticidal activity of the protein, and consequently increasing the insecticidal activity 30 and/or expression of the recombinant transgene in a plant cell. The amino acid changes